of resistance and study of risk factors for antibiotic resistance must be undertaken to elucidate reasons for high resistance.

http://dx.doi.org/10.1016/j.ijid.2014.03.619

Type: Poster Presentation

Final Abstract Number: 40.030
Session: Antibiotic Resistance
Date: Thursday, April 3, 2014
Time: 12:45-14:15
Room: Ballroom

Interdependence of multi-drug efflux pumps and quorum sensing systems in *Pseudomonas aeruginosa*

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Background: Multi-drug resistance (MDR) efflux pumps belonging to the nodulation cell division (RND) family in *Pseudomonas aeruginosa* are able to extrude out a wide variety of drugs and antibiotics. These complexes of inner-membrane, periplasm, and outer-membrane protein components are not merely molecular machines that pump out drugs. The very drugs that are pumped out by these efflux pumps can also induce expression of the efflux genes/proteins. In addition, the MDR efflux pumps transport out quorum-sensing molecules, which can induce expression of several genes including those related to biofilm formation, virulence, and efflux, thereby conferring additional resistance. Therefore, a complete understanding of the function of the MDR efflux pumps requires measurement and modeling of three processes: (a) how a given drug is extruded through these pumps; (b) how the same drug induces the expression of these pumps; and (c) how the release of quorum-sensing molecules cause the expression of biofilm, virulence, and efflux genes.

Methods & Materials: We measured and modeled the survival kinetics and intra- and extra-cellular concentration of ciprofloxacin in the wild-type *P. aeruginosa* strain PAO1 and the mutants deficient in efflux and quorum sensing. We also measured the formation of biofilm, antibiotic-induced expression of virulence, and efflux genes by real-time qPCR (Fluidigm) and modeled how the expression of these genes confers additional drug resistance *via* biofilm formation and enhanced virulence.

Results: We showed that (i) the *P. aeruginosa* tripartite MexA-MexB-OprM protein complex plays an important role in the efflux of ciprofloxacin and in the induction of the quorum-sensing pathways (ii) a sharp increase in the efflux within 5–10 minutes after the treatment of ciprofloxacin after which the efflux levels off (iii) the quorum sensing mediated biofilm, virulence, and efflux genes are expressed at higher level by mature biofilm than the nascent one providing additional resistance.

Conclusion: Combination of experimental and modeling studies provides an insight into how MDR efflux and quorum sensing systems work together to confer drug resistance. A similar approach can be extended to understand the structural, genetic, and cellular processes underlying the function of MDR efflux pumps.

http://dx.doi.org/10.1016/j.ijid.2014.03.620

Type: Poster Presentation

Final Abstract Number: 40.031
Session: Antibiotic Resistance
Date: Thursday, April 3, 2014
Time: 12:45-14:15
Room: Ballroom

Assessment of *Legionella pneumophila* antibiotic susceptibility

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Background: Routine susceptibility testing of *Legionella* spp. are not recommended because of the difficulty in determining standard minimal inhibiotry concentration value (MIC). It is associated with high nutrient requirements of these bacteria and inactivation of some antibiotics by charcoal contained in the substrate, which is necessary for cultivation of *Legionella* species. In Poland, susceptibility testing of environmental *Legionella* isolates has never been published, therefore the purpose of this study was to analyze the antimicrobial susceptibility of *L. pneumophila* strains isolated from the water supply system to antimicrobial agents to commonly used in therapy *Legionella* infections.

Methods & Materials: Twenty-eight isolates of *L. pneumophila* (12 – *L. pneumophila* SG 2-14, 16 – *L. pneumophila* SG 1). Susceptibility test was performed for 3 antimicrobials using the E-test method (bioMérieux, France). The tested antibiotics were azithromycin (range 0,016-256 μg/ml), ciprofloxacin (range 0,002-32 μg/ml), and rifampicin (range 0,002-32 μg/ml). The media used for susceptibility test was BCYE-α for *L. pneumophila*. Because there are no official guidelines for susceptibility testing for bacteria of the genus *Legionella*, breakpoints used were according to the literature data.

Results: Of all tested strains of *L. pneumophila* (n = 28), one was resistant to azithromycin. This was stain *L. pneumophila* SG 2-14 isolated from the water system in sanatorium. All isolates were found to be sensitive to ciprofloxacin and rifampicin. Only azithromycin-resistant strain exhibited much less sensitive to ciprofloxacin and rifampicin in comparison to the other tested *L. pneumophila*. The MIC_{50} for azithromycin, ciprofloxacin, and rifampicin were 0.032, 0.125, and 0.003 μg/ml, respectively.

Conclusion: Our study has shown resistance to azithromycin strain of *L. pneumophila* SG 1. This resistance mechanism is unknown and needs further study. It is possible that therapeutic failure in Legionnaires' disease may be associated with resistance and this should be taken into account. These data can be used as a reference for the detection of resistance in clinical *L. pneumophila* isolates and as a setting of clinical breakpoints. Ciprofloxacin and rifampicin have a good *in vitro* activity against *L. pneumophila* SG 1 and SG 2-14 in Poland.

http://dx.doi.org/10.1016/j.ijid.2014.03.621